

DTIC FILE COPY

②

SECURITY C

AD-A203 582

ATION PAGE

Form Approved
OMB No. 0704-C188

1a REPORT SECURITY CLASSIFICATION (U)		1b RESTRICTIVE MARKINGS	
2a SECURITY CLASSIFICATION AUTHORITY		3 DISTRIBUTION/AVAILABILITY OF REPORT Distribution Unlimited	
2b DECLASSIFICATION/DOWNGRADING SCHEDULE			
4 PERFORMING ORGANIZATION REPORT NUMBER(S) Massachusetts General Hospital		5 MONITORING ORGANIZATION REPORT NUMBER(S)	
6a NAME OF PERFORMING ORGANIZATION Massachusetts General Hospital	6b OFFICE SYMBOL (if applicable)	7a NAME OF MONITORING ORGANIZATION Office of Naval Research	
6c ADDRESS (City, State, and ZIP Code) Fruit Street Boston, MA 02114		7b ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000	
8a NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research	8b OFFICE SYMBOL (if applicable) ONR	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-88-K-0078	
8c ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000		10 SOURCE OF FUNDING NUMBERS	
		PROGRAM ELEMENT NO	PROJECT NO TASK NO 441m 804 WORK UNIT ACCESSION NO.
11 TITLE (Include Security Classification) (U) Adaptation and Regulation of the Transduction Mechanism in Vestibular Hair Cells.			
12 PERSONAL AUTHOR(S) Corey, David Paul/ Assad, John Abraham/ Hachohen, Nir/ Smith, Wendy Jane			
13a TYPE OF REPORT Annual	13b TIME COVERED FROM 12/87 TO 12/88	14 DATE OF REPORT (Year, Month, Day) 88/12/15	15 PAGE COUNT 5
16 SUPPLEMENTARY NOTATION			
17 COSATI CODES		18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	
		mechanoreceptor, adaptation, auditory system, ion channel	
		vestibular system, motility	
19 ABSTRACT (Continue on reverse if necessary and identify by block number)			
20 DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS		21 ABSTRACT SECURITY CLASSIFICATION (U)	
22a NAME OF RESPONSIBLE INDIVIDUAL Dr. Igor Vodyanov		22b TELEPHONE (Include Area Code) (202) 696-4056	
		22c OFFICE SYMBOL ONR	

DTIC
ELECTE
1989
S
E
D

ANNUAL REPORT

R&T CODE: 441m804---01

DATE: 15 DEC 1988

FIRST ANNUAL REPORT ON CONTRACT N00014-88-K-0078

PRINCIPAL INVESTIGATOR: David P. Corey

CONTRACTOR: Massachusetts General Hospital

CONTRACT TITLE: Adaptation and Regulation of the Transduction Mechanism in Vestibular Hair Cells

START DATE: 15 December 1987

→ The RESEARCH OBJECTIVES: To characterize the time and displacement dependence of adaptation in hair cells; to localize the site of action of calcium and determine its calcium specificity; to identify and localize calcium-binding and structural proteins in stereocilia that may mediate calcium's effect.

PROGRESS (Year 1): Work on this project has gone extremely well: the time and displacement dependence objective is done, and a manuscript has been submitted to the *Journal of Neuroscience*. The results indicate that the adaptation process involves the movement of the attachment point of the spring attached to transduction channels; this may be a movement of one end of the morphologically-defined "tip links". They also suggest that there are two separate processes in the adaptation: One is a slipping or relaxing process that is linearly proportional in rate to the displacement, indicating that the rate may depend on tension in the tip link. The other is a climbing or tensioning process that is largely independent of displacement, suggesting an intrinsic climbing rate. Incidentally, the rate and final tension of the element are nearly identical to that of myosin moving on actin.

We also have a good measure of the calcium dependence, using the microphonic preparation, and have made simple measurements of the cation specificity. Generally, the relaxation rate is increased by high calcium, but the tensioning rate is less sensitive. The binding site appears to be specific for calcium, as magnesium, strontium or barium inhibit calcium's action.

Or, another project, patch clamp experiments have suggested that the site of calcium action is inside the tips of the stereocilia (manuscript in press in *Proc. Natl. Acad. Sci*). While this should be confirmed with more direct methods, at this point all results are consistent with some molecular motor intimately associated with the transduction apparatus at the tips of the stereocilia.

→ Immunohistochemical experiments have indicated that calmodulin is inside the tips of the stereocilia, as well as in the cell body. There is also calbindin throughout the cell, based on immunohistochemistry. More calcium-binding proteins are being discovered every day, however, and it will be an interesting task to determine which of the many candidates is actually mediating the calcium dependence.

WORK PLAN (Year 2): We feel that a complete understanding of the adaptation process will only come about through an eventual identification and understanding of the specific proteins involved. Consequently, we will put a lot of effort into developing a method to purify stereocilia, and to separate the proteins in them with one- and two-dimensional gel electrophoresis. It may be possible to identify and localize these proteins with a combination of immunoblots and immunocytochemistry. The ultimate goal (which may not be within the scope of this grant), would be to determine the identity of a particular protein, localize it within the cilium, and understand its physiological role in the transduction or adaptation.

For year 2, then, efforts will focus on the biochemical separation and identification of stereociliary proteins, and on immunohistochemical localization with antibodies to known proteins. Physiology experiments will continue in order to finish up some of the first year objectives.

INVENTIONS (Year 1): While we have modified some standard techniques in novel ways, none would be considered patentable inventions.

PUBLICATIONS AND REPORTS (first year):

Assad, J.A., Hacohen, N., and Corey, D.P. (1989) Voltage dependence of adaptation and active bundle movement in bullfrog saccular hair cells. *Proc. Natl. Acad. Sci. USA* (in press).

Hacohen, N., Assad, J.A., Smith, W.J., and Corey, D.P. (1989) Regulation of tension on hair-cell transduction channels: Displacement and calcium dependence. *J. Neurosci.* (submitted)

TRAINING ACTIVITIES: John Assad, a graduate student in the Neurobiology Program at Harvard Medical School, has been working on the physiology of this project since its beginning. Gordon Shepherd and Philip Huang, both MD/PhD students at Harvard Medical School, have started to work on biochemical aspects of the project. All are exceptionally good and are key to the success of the project.

Women or minorities - 1
Non-citizens - 0

Accession For	
NTIS GR&I	<input checked="checked" type="checkbox"/>
DTIC TAB	<input checked="checked" type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By _____	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	



ADMINISTRATORS

Dr. Igor Vodyanoy, Code 1141SB (2 copies)
Scientific Officer, Biophysics
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

Administrator (2 copies)
(Enclose DTIC Form 50)
Defense Technical Information Center
Building 5, Cameron Station
Alexandria, VA 22314

James P. Foley
Contracts Administrator Dept of Navy
Resident Representative
Office of Naval Research
M.I.T. BLDG E-19-628
Cambridge, MA 02139-4309

Annual and Final Reports Only (one copy each)

Program Manager
Biological/Human
Factors Division
Code 125
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

Program Manager Defense
Technical Support Technology
Directorate
Office of Naval Technology
Code 223
800 N. Quincy Street
Arlington, VA 22217-5000

DoD ACTIVITIES

Commander
Chemical & Biological Sciences Div.
Research Army Research Office
P.O. Box 1221
Research Triangle Park, NC 27709

Code 40, Naval Medical
Research & Development Comm.
Naval Medical Command
National Capital Region
Bethesda, MD 20814-5044

Directorate of Life Sciences
Air Force Office of Scientific Res.
Bolling Air Force Base
Washington, DC 20332

Code 00, Naval Aerospace
Medical Institute
Naval Air Station Pensacola
Pensacola, FL 32508-5600

Code 00
Naval Aerospace Medical Research Lab.
Naval Air Station
Pensacola, FL 32508-5700

Code NMRDC-00
Naval Medical Research and
Development Command
Naval Medical Command
National Capital Region
Bethesda, MD 20814-5044

Douglas W. Call, CAPT, MSC, USN
Naval Biodynamics Laboratory
Box 29407, Michoud Station
New Orleans, LA 70189-0407

Naval Aerospace Medical Command/Code 23
Washington, DC 20372-5120

DISTRIBUTION LIST FOR REPORTS

VESTIBULAR TRANSDUCTION CONTRACTORS

Dr. Jonathan J. Art
Dept. of Pharmacological and
Physiological Sciences
The University of Chicago
947 East 58th Street
Chicago, IL 60637

Dr. Thomas P. Kerr
Department of Otolaryngology
Wayne State University
School of Medicine
540 East Canfield Avenue
Detroit, MI 48201

Dr. Chi-ming Huang
School of Basic Life Sciences
Univ. of Missouri-Kansas City
Kansas City, Missouri 64110-2499

Dr. Charles M. Oman
Department of Aeronautics &
Astronautics
Massachusetts Inst. of Technology
Cambridge, MA 02139

Dr. David P. Corey
Wellman 414
Massachusetts General Hospital
Fruit Street
Boston, MA 02114

Dr. Manning J. Correia
Department of Otolaryngology
and Physiology & Biophysics
University of Texas Medical Branch
Galveston, TX 77550

Dr. Ruth A. Eatock
Department of Physiology
University of Rochester
Box 642, 601 Elmwood Avenue
Rochester, NY 14642

Dr. Jay M. Goldberg
Department of Pharmacological and
Physiological Sciences
The University of Chicago
947 East 58th Street
Chicago, IL 60637

Dr. Stephen M. Highstein
Department of Otolaryngology
Washington University School of Medicine
724 South Euclid Avenue
St. Louis, MO 63110